GENETIC ANALYSIS OF THE CROSS BETWEEN THE SELF-FERTILE *NICOTIANA LANGSDORFFII* AND THE SELF-STERILE *N. SANDERAE*.

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(With Plate IV.)

Nicotiana Langsdorffii Weinm. in its typical form is characterised by green flower colour and complete lack of anthocyanin in the petals, by the dark colour of the anthers and the presence of blue pollen grains, and finally by its complete self-fertility. Other typical characters, such as the shape and size of the petals, will not be considered further. In the taxonomic literature several varieties differing from the type are described which are most probably the result of segregation after natural crossing.

N. Sanderae hort. is the collective name of a large number of garden forms which are probably segregates of crosses between two true species, *N. alata* and *N. Forgetiana*. The latter, according to the original description, is a plant with comparatively small scarlet flowers. The anthers are dark, but the pollen is yellowish white. It flowers by day, while *N. alata* is a night-flowering plant adapted to pollination by moths. The flowers of *alata* are very large. The petals are ivory coloured on the inner side and greenish magenta outside. As in *Forgetiana* the anthers are dark and the pollen yellowish white. Among the *Sanderae* types all possible combinations may be found, including plants with magenta petals or with an ivory colour over scarlet. The size of the flowers also varies widely. One "albino" variety is characterised by the complete absence of anthocyanin and related pigments in all organs of the plant, such as stem base, petals and anthers. The seeds, which in all the other forms are dark brown and round, are whitish yellow and wrinkled on white-flowered plants. All the *Forgetiana-Sanderae-alata* types, with only a very few exceptions, are self-sterile.

The inheritance of parasterility in *Sanderae* and of fertility in *Langsdorffii* has been very completely analysed (East and co-workers, 1915–33; Brieger, 1927, 1930 *b*; Anderson and de Winton, 1931). The

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analysis of the other characters is not yet very far advanced (cf. Matsuura, 1933). We shall refer to the publications later when presenting our results.

The chief object of the present paper is to show how far the segregation of colour factors, which are comparatively easily analysed, may be affected by gametic elimination due to linkage with the parasterility factors.

The experiments were started in 1926–7 and have been carried on since. Most of the results were obtained several years ago and published in preliminary communications (1929, 1930 *a*). The final publication, however, was held over for the appearance of a paper by East (1932) on similar material.

The plants were grown at the Kaiser Wilhelm Institut, Berlin-Dahlem, and my gratitude is due to the late Prof. Correns for giving me all facilities necessary. I wish also to thank Herr Inspektor Jenke for his careful supervision of my cultures.

Fertility *versus* parasterility.

The parasterility in *Nicotiana Sanderae* is due to the action of a series of multiple allelomorphs $\mathbf{S}_1$, $\mathbf{S}_2$, etc., acting in the following way (East and Mangelsdorf, 1925, 1926). Pollen tubes carrying an **S**-allele are not able to grow down a style carrying the same allele at a speed sufficient to allow of their effecting fertilisation. This inhibitive action may vary according to the presence of modifiers (Brieger, 1927, 1930 *b*; East and Yarnell, 1929) or to specific physiological conditions (end-season or bud pseudofertility according to East).

The fertility factor $\mathbf{S}_f$ of *Langsdorffii* has no such inhibiting effect. Furthermore, it does not affect the action of other **S**-alleles present in a diploid plant (East, 1932). A heterozygote $\mathbf{S}_f\mathbf{S}_1$, for instance, when selfed, produces only $\mathbf{S}_f\mathbf{S}_f$ and $\mathbf{S}_f\mathbf{S}_1$ plants, in equal numbers, owing to the elimination of all $\mathbf{S}_1$ pollen tubes. East proved this assumption mainly by using back-crosses. He gave no extensive data on selfed F_1 hybrids between *Langsdorffii* and *Sanderae*.

Our F_1 plants behaved as would be expected. After selfing old flowers (not buds) a majority of self-fertile plants was obtained. The few self-sterile offspring (less than 3 per cent., see Table I) can be explained in several ways. It is, for instance, possible that modifying factors were present, such as the hypothetical **A** factors of East (1932) which occasionally permitted a pollen tube carrying the sterility allele to accomplish fertilisation after selfing. Apart from this small number of self-

steriles obtained after selfing the F_1 the results are in accordance with those of East. The same is true concerning the inheritance of factors linked with the **S**-alleles, as we shall see later. One point, however, in East's experiments I was not able to verify.

East (1932) reports that in his crosses the parasterility factors in the plasma "largely derived from *Langsdorffii*" when homozygous produced male sterility. In my material the number of male steriles was extremely low, and not higher than in pure *Sanderae* crosses. The frequency was not connected with any particular cytoplasm.

TABLE I.

$F_1 \frac{S_x}{S_f}$ *selfed.* Langsdorffii *cytoplasm.*

Family	Self-fertile	Self-sterile	Without pollen
HA 27	41	4	1
HD 27	66	1	—
HE 27	53	1	—
HG 27	21	—	2
HH 27	27	—	—
Total	208	6	3

THE WHITE FLOWER FACTOR, **c**.

Brieger and Mangelsdorf (1926, 1927) have shown that the absence of anthocyanin and related pigments in some *Sanderae* lines is due to a single recessive factor **c**. The segregation of **Cc** heterozygotes is quite normal. I have found the same again in the back-crosses of *Langsdorffii-Sanderae* hybrids heterozygous for this factor, the former species being always **CC**. Using the heterozygotes as females, I obtained good agreement with expectation both in the results of individual families and in the total (49·5 per cent. in 495 plants, cf. Table II). In the reciprocal back-cross, however, there is always a slight excess of the recessive type, both in individual families and in the total (57 per cent. in 361 plants, cf. Table III). The deviation is just a little smaller than three times the standard error (2·78). But as all families deviate in the same direction this small deviation is statistically significant. A possible explanation is that in the heterozygote used as male parent there is slight pollen tube competition. The S_f pollen tubes which carry the linked dominant gene **C** in 80 per cent. may grow just a little slower than the pollen tubes carrying the S_x allele and the **c** factor from *Sanderae*.

In another set of crosses involving mainly *Sanderae* families I found, however, a much higher deviation from expectation. A fully self-sterile red-flowered line corresponding to *N. Forgetiana* was crossed with a

TABLE II.

$$\frac{S_F\,C\,B_1}{S_1\,c\,b_1}\,\frac{i\,B_2}{I\,b_2}\,\frac{G}{g} \times \frac{S_x\,c\,b_1}{S_y\,c\,b_1}\,\frac{I\,b_2}{I\,b_2}\,\frac{g}{g}.$$ Sanderae *cytoplasm.*

Petals ...	With anthocyanin						White								
Pollen ...	Blue			White			Blue			White					
Petals	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Total	% white petals	% white pollen	% non-green petals
N 795	13	15	*28*	12	29	*41*	—	—	—	31	38	*69*	138	50·0	79·7	59·4
N 796	11	16	*27*	10	29	*39*	1	—	*1*	23	35	*58*	125	47·2	77·6	64·0
N 801	10	20	*30*	4	22	*26*	—	—	—	22	34	*56*	112	50·0	73·5	76·8
N 802	15	15	*30*	3	25	*28*	—	—	—	34	28	*62*	120	51·7	68·2	56·7
Total	49	66	*115*	29	105	*134*	1	—	*1*	110	135	*245*	495	49·5	76·2	61·5
% found			23·2			27·1			—			49·5		—	—	—
% expected			25·0			25·0			—			50·0		50·0	75·0	50·0
% standard error			1·95			1·95			—			3·56		3·5	1·9	3·5
% deviation			−1·8			+2·1			—				−0·5	−0·5	+1·2	+11·5

TABLE III.

$$\frac{S_x\,c\,b_1}{S_y\,c\,b_1}\,\frac{I\,b_2}{I\,b_2}\,\frac{G}{g} \times \frac{S_F\,C\,B_1}{S_1\,c\,b_1}\,\frac{i\,B_2}{I\,b_2}\,\frac{g}{g}.$$ Sanderae *cytoplasm.*

Petals ...	With anthocyanin						White								
Pollen ...	Blue			White			Blue			White					
Petals	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Total	% white petals	% white pollen	% non-green petals
N 797	5	8	*13*	1	12	*13*	—	1	*1*	24	20	*44*	71	63·4	80·3	57·7
N 798	8	9	*17*	5	18	*23*	—	—	—	33	29	*62*	102	60·8	83·3	54·9
N 803	14	15	*29*	6	11	*17*	—	—	—	25	23	*48*	94	51·1	69·2	52·1
N 804	16	12	*28*	8	15	*23*	—	—	—	14	39	*53*	104	51·0	73·1	63·5
Total	43	44	*87*	20	56	*76*	—	1	*1*	96	111	*207*	371	57·6	78·2	58·5
% found			23·5			20·5			—			55·8				
% expected			25·0			25·0			—			50·0		50·0	75·0	50·0
% standard error			2·25			2·25			—			2·60		2·6	2·2	2·6
% deviation			−1·5			−4·5			—			+5·8		+7·8	+3·2	+8·5

white-flowered *Sanderae* which was also self-sterile, but was easily selfed in the bud. All the F_1 plants were red-flowered. As they all came from white seeds, the white-flowered plant having been used as the mother, they must all have been hybrids and have had the constitution **Cc**.

These hybrids gave, after bud selfing, not 25 per cent. of white-flowered plants as expected, but 55·5 per cent. (33 in 59 plants). Here again pollen-tube competition may be the cause of the excess. The **S**-allele which was linked with the **C** factor came from the completely self-sterile parent, and pollen tubes carrying this particular **S** gene may be slower than those carrying the factor from the pseudofertile parent. Assuming that only the latter accomplished fertilisation and that crossing-over between **S** and **C** amounted to 20 per cent., we should expect about 40 per cent. white-flowered plants. We have found a higher percentage, but the numbers are too small to decide whether the deviation is significant.

The back-cross data show that pollen-tube competition cannot be the only disturbing factor. Using the heterozygote as female, only two out of five families gave as expected 50 per cent. white-flowered plants (50 in 98, 55 in 109). Two other families gave no whites at all (in 104 and 110), and one gave about 3 per cent. (5 in 131). In the reciprocal cross two families gave about 14 per cent. of recessives (5 in 53, 9 in 62). I am quite unable to explain these peculiar results but hope that further experiments may lead to a solution.

THE IVORY FLOWER COLOUR, **i**.

The ivory factor of *alata* inhibits the development of anthocyanin on the upper (inner) surface of the petals. The stem, and also the outer surface of the petals, may develop anthocyanin in varying degree, depending on other factors present and external conditions. The dominant gene **I** permits the full development of pigment. The seeds are always brown and round.

The segregation of this factor pair is normal. In the data of Brieger and Mangelsdorf there was always a small deficit of ivory plants (44·7 per cent. instead of 50 per cent. in 114 back-crosses plants and 17·3 per cent. instead of 25 per cent. in 203 F_2 plants). This deficit is not found in the families reported here. Four back-cross families (Table IV) gave a total of 350 coloured and 323 ivory plants (48·0 per cent.), and two F_2 families, expected to give a 3 : 1 segregation, gave 81 ivory plants in 305 plants, or 26·6 per cent.

The **P** factor of East (1932) and the **F** factor of Skalinska (1921) may be identical with **I**.

Simultaneous segregation for white and ivory.

A distinction between white and ivory plants is generally possible, the former having green buds while in the latter the tips of the buds are generally brownish (anthocyanin plus chlorophyll). But in cases in which this character is not very clearly expressed, the types can be distinguished only by the colour and shape of the seeds: white angular *versus* brown round. However, in plants with blue pollen even this distinction cannot be used. All plants with blue pollen have round brown seeds regardless of whether they carry the gene **C** or are homozygous **cc**. Therefore in families segregating for both factors **I/i** and **C/c** and the pollen colour, I have classified the recessive types **ii C**, **I cc** or **ii cc** all in one group "white."

In families where the two types could be distinguished it is evident that **cc** in suppressing the formation of anthocyanin is epistatic to all other anthocyanin factors, including **i** or **I**.

In Table VII the results of selfing doubly heterozygous F_1 plants are summarised. 25 per cent. of the offspring should be **ii**. The segregation of the **C/c** factors is, however, disturbed by the eliminating action of the self-sterility gene coming from the self-sterile *Sanderae* parent, which is linked in those plants with the recessive **c** allele. Of the functioning $\mathbf{S_1}$ pollen tubes, 80 per cent. carry the **C** and 20 per cent. the **c** allele. Half of the latter should fertilise **C** eggs and the other half **c** eggs. The expected percentage of **cc** plants after selfing an old flower would then be 10 per cent. Taking into consideration the fact that 25 per cent. of the **C** plants will be **ii**, we should expect 32·5 per cent. "white"-flowered plants.

The numbers actually found are much smaller, and in the total the minus deviation exceeds four times the standard error. From the data concerning inheritance of pollen colour it seems that this deficiency is due to the segregation of the **I/i** genes and not to the **C/c** factors.

Another cross represents a back-cross for the **C/c** genes without any elimination, and an F_2 for the ivory gene (Table VI). 50 per cent. of the offspring should therefore be **cc** and of the rest one-quarter or 12·5 per cent. of the total should be **ii**. The total expectation of white-flowered plants amounts to 62·5 per cent. The numbers found agree very well with this expectation, the deviation being smaller than the standard error.

TABLE IV.

$$\frac{S_F\,C\,B_1}{S_1-b_1}\,\frac{i\,B_2}{I\,b_2}\,\frac{G}{g}\times\frac{S_x\,C\,b_1}{S_y\,C\,b_1}\,\frac{i\,b_2}{i\,b_2}\,\frac{g}{g}.$$ Sanderae *cytoplasm.*

Petals ...	With anthocyanin						Ivory									
Pollen ...	Blue			White			Blue			White						
Petals	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Total	% ivory petals	% white pollen	% non-green petals
N 782	4	4	*8*	36	53	*89*	5	5	*10*	41	31	*72*	179	46·0	90·3	51·0
N 787	3	4	*7*	62	41	*103*	16	24	*40*	9	37	*46*	196	43·9	76·0	54·0
N 793	8	1	*9*	50	15	*65*	20	22	*42*	25	22	*47*	163	54·6	68·7	36·8
N 799	1	12	*13*	28	28	*56*	15	18	*33*	13	20	*33*	135	48·9	65·8	57·8
Total	16	21	*37*	176	137	*313*	56	69	*125*	88	110	*198*	673	48·0	79·5	52·4
% found			5·50			46·5			18·6			29·4				
% expected			2·5			47·5			22·5			27·5		50·0	75·0	50·0
% standard error			0·60			1·73			2·59			2·16		1·9	1·7	1·9
% deviation			3·0			1·0			3·9			1·9		−2·0	+4·5	+2·4

TABLE V.

$$\frac{S_f\,C\,B_1}{S_x\,C\,b_1}\,\frac{i\,B_2}{I\,b_2}\times\frac{S_y\,c\,b_1}{S_2\,c\,b_1}\,\frac{i\,b_1}{I\,b_1}.$$ Sanderae *cytoplasm.*

Petals ...	With anthocyanin						Ivory									
Pollen ...	Blue			White			Blue			White						
Petals	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Total	% ivory petals	% white pollen	% non-green petals
N 783	12	8	*20*	37	62	*99*	5	5	*10*	13	15	*28*	157	24·2	80·9	57·3
N 788	6	14	*20*	26	59	*85*	10	10	*20*	7	16	*23*	148	29·0	73·0	66·9
Total	18	22	*40*	63	121	*184*	15	15	*30*	20	31	*51*	305	26·6	77·0	62·0
% found			13·1			60·4			9·8			16·7				
% expected			15			60·0			10·0			15·0		25·0	75·0	50·0
% standard error			2·8			2·8			1·7			2·0		2·48	2·48	2·86
% deviation			−1·9			+0·4			−0·2			+1·7		+1·6	+2·0	+12·0

TABLE VI.

$$\frac{S_f\ C\ B_1}{S_1\ c\ b_1}\ \frac{i\ B_2}{I\ b_2}\ \frac{G}{g} \times \frac{S_x\ c\ b_1}{S_y\ c\ b_1}\ \frac{i\ b_2}{I\ b_2}\ \frac{g}{g}.$$ Sanderae *cytoplasm.*

Petals ...	With anthocyanin						Ivory or white									
Pollen ...	Blue			White			Blue			White				% white petals	% white pollen	% non-green petals
Petals	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Total			
N 794	13	15	*28*	4	19	*23*	10	7	*17*	41	34	*75*	143	64·3	68·5	52·4
N 800	6	6	*12*	9	30	*39*	9	5	*14*	34	36	*70*	135	62·1	80·7	57·0
Total	19	21	*40*	13	49	*62*	19	12	*31*	75	70	*145*	278	63·3	74·5	54·7
% found			14·4			22·3			11·1			52·2		—	—	—
% expected			15·0			22·5			10·0			52·5		62·5	75·0	50·0
% standard error			2·1			2·5			1·8			2·9		2·90	2·60	2·99
% deviation			−0·6			−0·2			+1·1			−0·3		+0·8	−0·5	+4·7

TABLE VII.

$$\frac{S_f\ C\ B_1}{S_x\ c\ b_1}\ \frac{i\ B_2}{I\ b_2}\ \frac{G}{g}.$$ *Selfed open flower. No.* 781, Sanderae *cytoplasma; Nos.* 104–108, Langsdorffii *cytoplasm.*

Petals ...	With anthocyanin						Ivory or white									
Pollen ...	Blue			White			Blue			White				% white petals	% white pollen	% non-green petals
Petals	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Green	Non-green	Total	Total			
N 104	54	16	*70*	36	5	*41*	11	4	*15*	22	4	*26*	152	27·0	44·1	10·1
N 105	50	13	*63*	30	3	*33*	5	7	*12*	20	5	*25*	133	27·8	43·6	20·6
N 107	9	1	*10*	4	1	*5*	1	1	*2*	2	2	*4*	21	—	—	—
N 108	13	6	*19*	4	0	*4*	1	1	*2*	2	0	*2*	27	—	—	—
Total	126	36	*162*	74	9	*83*	18	13	*31*	46	11	*57*	333	26·4	42·0	20·7
N 781			*37*			*13*			*12*			*1*	63	20·6	22·2	—
Total			*199*			*96*			*43*			*58*	396	—	—	—
% found			50·3			24·2			10·9			14·6		25·5	38·8	(20·7)
% expected			45·9			21·6			21·6			10·9		32·5	32·5	25·0
% standard error			2·5			2·1			2·1			1·9		2·35	2·35	2·44
% deviation			+4·4			+2·6			−10·7			+3·7	−7·0	+6·3	−4·3	

GREEN FLOWER COLOUR.

While the flowers of *Langsdorffii* are green, owing to a high content of chlorophyll, the flowers of the *Sanderae* types contain when fully opened very little if any green pigment at all in the petals. The segregation for this character is not easily followed, on account of difficulties of classifica-

TABLE VIII.

$\frac{S_1\ C\ B_1}{S_1\ C\ b_1}\ \frac{i\ B_2}{I\ b_2}$. *Selfed in bud.* Sanderae *cytoplasm.*

Petals ...	Coloured		White			% white	% white
Pollen ...	Blue	White	Blue	White	Total	flower	pollen
N 395 = 1 N 215 selfed	35	2	5	6	48	26·1	17·4
N 399 = 3 N 215 selfed	34	6	6	9	55	27·3	27·3
N 404 = 4 N 215 selfed	26	49	6	0	81	7·4	60·5
N 382 = 1 N 216 selfed	44	36	18	0	98	18·4	37·8
N 385 = 4 N 217 selfed	37	39	16	2	94	19·2	43·6
N 405 = 3 N 217 selfed	21	30	9	13	73	27·4	57·5
Total found	197	162	60	30	449	—	—
% found	43·6	36·2	13·4	6·7		20·1	42·7
% expected	38·3	36·7	18·0	7·0		25·0	43·7
% standard error	2·3	2·3	1·8	1·2		2·0	2·3
% deviation	+5·3	−0·5	−4·6	−0·3		−4·9	−1·0

TABLE IX.

Segregation in back-crosses of Gg *to* gg. *Cytoplasm from* Sanderae.

F_1 plant used as	Number in family	% non-green	Deviation From 50 %	Deviation From 57·8 %
7 ♀♀ 3 ♂♂ ♀	163	36·8	−13·2	−21·0
♀	179	51·0	+ 1·0	− 6·8
♂	94	52·1	+ 2·1	− 5·7
♀	143	52·4	+ 2·4	− 5·4
♀	196	54·0	+ 4·0	− 3·8
♂	102	54·9	+ 4·9	− 2·9
♀	120	56·7	+ 6·7	− 1·1
♀	135	57·0	+ 7·0	− 0·8
♀	157	57·3	+ 7·3	− 0·5
♂	71	57·7	+ 7·7	− 0·1
5 ♀♀ 1 ♂ ♀	135	57·8	+ 7·8	+ 0·1
♀	138	59·4	+ 9·4	+ 1·6
♂	104	63·5	+13·5	+ 5·7
♀	125	64·0	+14·0	+ 6·2
♀	148	66·9	+16·9	+ 9·1
♀	112	76·8	+26·8	+19·0
Total found	2122	57·8 %	+ 7·8 %	
Expected		50·0 %	(± 1·17)	

tion. In the coloured types it is especially difficult to distinguish flowers with much chlorophyll from those with little or none. In the white and ivory plants the distinction is generally easy.

The results of 16 back-cross families are given in Table IX. In all but one family the recessive type without chlorophyll in the petals is

significantly in excess. In the total of 2122 plants 57·8 per cent. are non-green, instead of 50 per cent. This deviation is about seven times the standard error. If we calculate the deviations from the value actually found for all families (*i.e.* 57·8 per cent.), the distribution of plus and minus deviations is fairly normal.

The situation becomes still more complicated if we consider the four flower types separately. Adding the data in Tables II, III, IV and VI we find in the classes with anthocyanin in the petals, and blue pollen, 55·4 per cent. of 314 plants to be non-green, in the class "white or ivory" + blue pollen, 52·6 per cent. of 188 plants and in the class "white or ivory" + white pollen the results are 54·0 per cent. in 846. The excesses are small but always tend in the same direction. The fourth class with full-coloured flowers and white pollen gave an excess which is, however, of a very different magnitude, 76·8 per cent. in 609 plants being non-green. This excess is about 13 times the standard error.

These aberrations cannot be connected with pollen tube competition because the deviations were found regardless of whether the heterozygotes were used as males or females (cf. Table IX).

In the few F_2 families analysed there is always a deficiency in the green class, not an excess. In the total of 336 plants 20·5 per cent. were non-green instead of 25 per cent., and in the largest family of 152 plants only 10·0 per cent. green plants were found. Here again the deviation in the full colour+white pollen class is the biggest, while the other three types segregate almost normally into 3 green to 1 non-green.

I am unable to explain these deviations. I think, however, one is justified in assuming that one main dominant factor **G** causes the development of chlorophyll to its full extent; but one cannot at present say whether special modifiers or complicated gene interactions cause the deviations in general, and especially the different segregation in the four flower types. Cytoplasmic influence can at present be neither proved nor disproved. Incidentally all back-cross families had *Sanderae* cytoplasm, while the F_2 families had *Langsdorffii* plasma. Further experiments are in progress to make this point clear.

The inheritance of the green pigment in the *Langsdorffii-Sanderae* crosses has been studied by Skalinska and by East. Skalinska (1921) found a more complex segregation. It cannot be doubted that there exist several modifying factors. I was, however, unable to separate more than the main types, very green *versus* non-green, in the segregating families.

East (1932) also states that there is one main dominant factor present,

and his totals give a fairly good monofactorial segregation ($F_1 \times$ *Sanderae* 146 green : 190 not green, *Sanderae* $\times F_1$ 101 green : 87 not green). The individual data of his families, ranging from about 19 to 66 and mostly numbering about 40 plants, are used as the basis of a factorial scheme involving three factor pairs and assuming also an influence of the *Sanderae* cytoplasm. No conclusive statistical proof of these assumptions can be obtained from the small data. In his earlier publication (1916) East found in each of four F_2 families a deficit of recessive non-green plants as compared with the expectation of 25 per cent.: 23·7 per cent. in 257 plants, 23·1 in 65, 21·1 in 72 and 7·9 in 76 plants. One F_3 family gave 20·6 per cent. in a total of 141 plants. Thus East's and my data agree exactly. There was always a deficit of the recessive type after selfing heterozygotes, while back-crosses gave mostly an excess and only twice in 18 families a deficit.

The F_2 data reported by E. Anderson and de Winton (1931) correspond to a normal 3 : 1 ratio (36 : 11).

THE RED FLOWER COLOUR, **R**.

The red colour of *N. Forgetiana* is mainly due to one dominant gene **R**. The recessive gene **r** is present in all other *Sanderae* types and in *Langsdorffii*, and causes a magenta flower colour. Both genes are effective only in presence of the two basic factors **C** and **I**.

The segregation is not always simple. The colour of the F_1 plants may vary according to external conditions. The flowers of the plants grown in July in the field are always deep red like the *Forgetiana* parent. But in the greenhouse, and also apparently out of doors, under the influence of lower temperature the colour is more or less intermediate between magenta and red. It is therefore not astonishing that the scoring of F_2 families is not always possible.

Out of 14 back-cross families seven gave a normal monohybrid segregation, 317 plants being red-flowered and 307 magenta-flowered. Four families could not be scored, all 448 plants having different shades of magenta-red. The last three families gave results which correspond rather to a dihybrid ratio. Forty-one plants had scarlet flowers and 123 magenta flowers. All these families were grown under normal conditions in summer and out of doors.

The different type of segregation, mono- or dihybrid, must be due to genic causes. All plants were grown under conditions most favourable to the maximum development of the dominant scarlet colour, *i.e.* out of doors in summer. Furthermore the cytoplasm did not have any effect.

Two of the families giving a 1 : 1 ratio and one giving a 1 : 3 segregation had the cytoplasm of the red-flowered parent. All the remaining 11 families had the plasma of the magenta parent.

Blue pollen, $\mathbf{B_1B_2}$.

The blue pollen colour of *Langsdorffii* is due to two independent dominant factors. Both must be present in order to show any effect. Plants which have only one or neither of these dominant factors have white pollen. The pollen colour is due to a coloration of the exine and is a sporophytic character.

This factorial scheme is proved by the result of back-crossing doubly heterozygous F_1 plants to homozygous recessive *Sanderae* plants. In a total of 2122 plants 76·7 per cent. had white pollen. The deviation from the expected 75 per cent. is only twice the standard error and therefore not significant. No difference between the two types of back-crosses could be detected, as will be seen from Table IX.

The segregation in F_2, however, did not give such entirely satisfactory results. After selfing in the bud (Table VIII) the total of the six families (447 plants) gave 42·7 per cent. plants with white pollen. This represents only a slight deviation of 0·9 per cent. from the expected 9 : 7 ratio. But the data of the individual families show very divergent results. Only two families agree with expectation (in 98, 37·8 per cent., and in 94, 43·6 per cent. white pollen). Two show a great excess, which goes beyond the statistical limit of three times the standard error (in 73, 57·5 per cent. and in 81, 60·5 per cent. white pollen). The last two families show a great deviation in the other direction (in 55, 27·3 per cent. and in 46, 17·4 per cent.). The fact that two families show a significant excess of recessives and two others a deficiency of the same magnitude makes it very probable that both deviations are due to the same factor linked with one of the **B** loci. The segregating families in this case were actually not F_2 generations of *Sanderae* × *Langsdorffii*. F_2 plants carrying the dominant genes were back-crossed to the recessives, and double heterozygotes of these back-cross families were selfed. The back-crosses themselves showed that two of the F_2 plants selected were homozygous for one of the **B** factors and gave only a 1 : 1 segregation (44 blue : 57 white; 37 blue : 51 white), and that one was a double heterozygote and gave a 1 : 3 ratio (21 blue : 91 white).

One of the **B** factors ($\mathbf{B_1/b_1}$) is, as we shall see later, very closely linked to the **C/c** factor pair. It is, therefore, also linked with **S** factors and must give about 20 per cent. crossing-over with them. When

Langsdorffii-Sanderae F_2 plants are selfed in the open flower, the eliminating effect of the sterility genes must also affect the segregation of the pollen colour factor. The dominant gene $\mathbf{B}_1$ being coupled with the fertility gene $\mathbf{S}_f$ we expect all but the 20 per cent. cross-over tubes to carry this dominant gene. 90 per cent. of the offspring, therefore, obtain the $\mathbf{B}_1$ gene, and only 10 per cent. are $\mathbf{b}_1\mathbf{b}_1$. Of the first group $\frac{3}{4}$ have the $\mathbf{B}_2$ factor and $\frac{1}{4}$ $\mathbf{b}_2\mathbf{b}_2$ (22·5 per cent.). 32·5 per cent. of the total offspring fail to receive both dominant factors and are, therefore, characterised by white pollen. The total results of all five families (Table VII) are again in fair agreement with this expectation, 38·6 per cent. in 399 plants having white pollen. The deviation is just a little smaller than three times the standard error (6·1 per cent. against $3 \times 2{\cdot}2$ per cent.). Of the individual families three gave an excess of recessives and show nearly the ratios expected with no elimination due

TABLE X.

Inheritance of blue pollen colour in back-crosses of $\frac{\mathbf{B}_1}{\mathbf{b}_1}\frac{\mathbf{B}_2}{\mathbf{b}_2}$ *to* $\frac{\mathbf{b}_1}{\mathbf{b}_1}\frac{\mathbf{b}_2}{\mathbf{b}_2}$.

All in Sanderae *cytoplasm.*

F_1 plant used as		n	% white	Deviation
6 ♀♀ 2 ♂♂	♀	135	65·8	− 9·2
	♀	120	68·2	− 6·8
	♀	143	68·5	− 6·5
	♀	163	68·7	− 6·3
	♂	94	69·2	− 5·8
	♀	148	73·0	− 2·0
	♂	104	73·1	− 1·9
	♀	112	73·5	− 1·5
6 ♀♀ 2 ♂♂	♀	196	76·0	+ 1·0
	♀	125	77·6	+ 1·6
	♀	138	79·7	+ 3·7
	♂	71	80·3	+ 5·3
	♀	135	80·7	+ 5·7
	♀	157	80·9	+ 5·9
	♂	102	83·3	+ 8·3
	♂	179	90·2	+15·2
Total found		2122	76·7	+ 1·7
Expected			75·0	(± 0·84)

to linkage with the **S** factors (in 152, 44·1 per cent.; in 21, 42·9 per cent.; in 136, 42·7 per cent.). The other two give deficient ratios (in 22, 22·2 per cent.; in 63, 22·2 per cent.). It will be seen that here again, as in the families obtained after bud pollination, the families giving an excess and those giving a deficient ratio compensate each other and in the total give a statistically satisfactory result. One would seem to be justified in assuming in both cases the same source for the deviations, *i.e.* one factor linked with one of the **B** loci and favouring the appearance of recessives.

The segregation of pollen colour in this cross was first observed by Lock (1909), but his figures are much too small to be of any use. The F_2 figures published by East (1916) show, as compared with ours, a great excess of blue-pollen plants. East obtained in F_2 342 blue and 100 white pollen plants. If the pollinations of the F_1 plants were made in the bud stage, thus avoiding the selective action of the **S** factors, we should expect a 9 : 7 ratio. If, on the other hand, old flowers were selfed we should obtain approximately a 2 : 1 ratio.

Recently East (1932) arrived at the following interpretation: "Blue pollen is due to the interaction of three independent complementary factors **CDB**. The factor **D** (with **C**) apparently does not produce blue pollen." The actual data of East, however, do not confirm this trifactorial assumption. Furthermore it is not clear why the expected values "were calculated by using cross-over ratios" which were found for the linkage **S**–**C**, the heterozygotes were used as females in backcrosses. The numbers found, 43 blue : 146 white pollen plants, agree very well with our bifactorial scheme, which should give a 1 : 3 ratio.

In the reciprocal cross, *Sanderae* × F_1, East "again found some confusion. In certain combinations where $\mathbf{S}_f$ and one or perhaps two of the **A** factors (assumed to affect the expression of parasterility) are united, and the cytoplasm comes from *N. Sanderae*, there is a great excess of light anthers and blue pollen" (p. 197). No figures are given which show the actual amount of this excess. I have not found such protoplasmic influence. The majority of all such segregating families had the *Sanderae* cytoplasm and gave, as seen above, as good ratios as the F_2 families with *Langsdorffii* plasma.

Crassa leaf.

In several of the *Langsdorffii-Sanderae* F_2 families plants occurred which were characterised by a very peculiar type of growth (Figs. 1 and 2). The leaves were very narrow, strap-like, and formed a rosette out of which generally arose only a short stem with a limited number of flowers. The growth of these plants was very slow. Crassa plants very often die before flowering, and need as a rule about 10 months before reaching maturity.

Crassa plants selfed breed true for this type of growth. There was, however, a striking recovery after consecutive selfing. Some plants of the later inbred generation became somewhat similar to the normal plants (Fig. 2). This point is, however, still under investigation.

In crosses with normal unrelated plants the normal type of growth was always dominant. After selfing, these hybrids gave in four families

522 normal and 56 crassa plants. Four back-cross families using the same F_1 hybrids consisted of 351 normal and 106 crassa plants. Assuming full viability of the crassa types and a monofactorial segregation, we should expect after selfing 174 crassa plants to 522 normals, and after back-crossing 351 : 351. The actual figures found represent 32·2 per cent. of the expected value in the case of selfing and 30·2 per cent. in the case of back-crossing. The deviations are of the same magnitude in both types of segregating families and are most probably due to the reduced viability of the crassa type.

The data seem to show that the crassa type is due to one recessive gene $\mathbf{c}_R$, which greatly reduces viability in addition to causing the peculiar type of growth.

It is, however, remarkable that this recessive gene which has occurred several times independently in F_2 families of the *Langsdorffii-Sanderae* cross, but has never been observed in pure *Langsdorffii* or *Sanderae* lines. Something special in these hybrids must be responsible for the occasional occurrence of this mutation. Seeing that this type as compared with the normal is only characterised by negative properties, reduced vitality and abnormal growth, it may be due not to a new recessive gene but possibly to a chromosome aberration. Experiments are in progress to elucidate this point.

LINKAGES.

The *first linkage* reported in *N. Sanderae* was the one between the self-sterility factors and the basic colour factor **C**/**c** (Brieger and Mangelsdorf, 1926, 1927). The recombination percentage is on an average about 20 per cent. The same linkage relation was found by East (1932) for the *Langsdorffii-alata* cross. The present data are also in agreement.

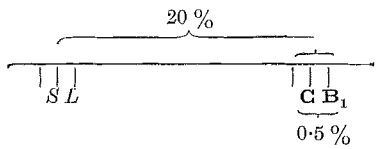
One of the pollen colour factors ($\mathbf{B}_1/\mathbf{b}_1$) belongs to the same linkage group. The possibility of such a linkage group occurring was pointed out by Anderson and de Winton (1931) and by East (1932). Our back-cross data furnish conclusive evidence (Tables II and III). In these back-crosses, $\frac{\mathbf{CB}_1}{\mathbf{cb}_1}\frac{\mathbf{B}_2}{\mathbf{b}_2} \times$ recessive, the determination of the recombination percentage is fairly simple. Half of all plants with the factor $\mathbf{B}_1$ have only blue pollen, owing to the segregation of the $\mathbf{B}_2/\mathbf{b}_2$ factor pair. Thus among the coloured offspring having the constitution **Cc** the deviation from the 1 : 1 segregation only can be used for the calculation of the linkage. The actual figures show a very good 1 : 1 segregation and indicate that the linkage between **C** and $\mathbf{B}_1$ must be very close. In the

white-flowered class all non-cross-over plants $\left(\frac{\mathbf{cb_1}}{\mathbf{cb_1}}\right)$ must have white pollen, and half of the cross-over plants also have white pollen, the other half blue pollen. In the total of 856 white-flowered plants only two had blue pollen. The linkage is very close and the recombination percentage is thus found to be only 0·5 per cent.

East (1932) found that the lethal effect originally connected with the $\mathbf{S_3}$ factor (East and Mangelsdorf, 1926) was due to a factor (l) very closely linked with the sterility gene, as first suggested by Brieger (1930 *b*).

Finally, Anderson and de Winton (1931) have shown that at least one of the factors responsible for the difference in flower size between *alata* and *Langsdorffii* is located in the same chromosome. Owing to the polyfactorial segregation the calculation of the recombination percentages was not possible.

In the map of the first chromosome of *N. Sanderae* therefore we have located four factors: the **S** genes and the lethal factor **l** very closely linked with **S**, the **C**/**c** factor pair and again very closely linked one of the factors for blue pollen colour. Owing to the closeness of the linkages it has not yet been possible to determine the exact sequence of the genes.



The *second linkage* group is so far represented by three factor pairs.

The ivory factor is linked with the second factor responsible for the blue pollen colour, $\mathbf{B_2}/\mathbf{b_2}$. The calculation of the recombination percentage is somewhat complicated by the segregation of the blue pollen factor $\mathbf{B_1}/\mathbf{b_1}$ and further by the fact that in our crosses the two factors are repelled. The **i** gene from *Langsdorffii* occurs with the dominant $\mathbf{B_2}$ gene. Assuming complete linkage, all homozygous ivory plants should have the $\mathbf{B_2}$ factors, but owing to the segregation for $\mathbf{B_1}/\mathbf{b_1}$ only half of these ivory plants show blue pollen. The excess of the white over the blue pollen class is due to crossing over between **i** and $\mathbf{B_2}$. From Table IV we get in this class the percentages for the individual families and the total shown below.

In the class of plants with coloured flowers (**Ii**) all plants should have ivory flowers except for the cross-overs between **I** and $\mathbf{b_1}$. Half of these cross-overs again will have white pollen and only half have both dominant

factors $\mathbf{B}_1$ and $\mathbf{B}_2$. The recombination percentage in this class is twice the actual percentage of blue pollen plants.

We can then calculate the following percentages:

	Family numbers				
Ivory class:	N 782	N 787	N 793	N 799	Total
No. in family	82	86	89	66	323
Percentage recessive	75·6	7·0	5·1	0	22·6
Magenta class:					
No. in family	97	110	74	69	350
Percentage recessive	16·5	12·7	24·3	34·7	21·1

The recombination percentage in the total is approximately 22 per cent.[1]

The crassa factor is most probably also located in the same chromosome. The data are not fully conclusive, as in the back-cross families of double heterozygotes **Ii** $\mathbf{C_r c_r}$ to the double recessives I have so far only been able to score the normal $\mathbf{C_r c_r}$ offspring. In these back-crosses we should expect half of the normal offspring to have coloured flowers and the other half to have ivory flowers if there were no linkage, or only plants with coloured flowers if the linkage were absolute. In the total of 319 plants 29·5 per cent. had ivory-coloured flowers representing the recombination value for the linked factors **I** and $\mathbf{C_r}$. The data in the individual families were very variable: 42·9 per cent. in 63 plants, 33·8 per cent. in 136 plants, 14·9 per cent. in 114 plants, and four plants in six.

The results of F_2 families show deviations from 25 per cent. which are of the order expected, with a percentage of recombination of about 30 per cent. In the total of 421 plants 13·1 per cent. had ivory flowers as compared with 9 per cent. expected. The individual families again show a wide variation; 22·7 per cent. in 88 plants, 14·1 per cent. in 118, 10·5 per cent. in 114, 5 per cent. in 101.

It would seem to be justifiable to accept the assumption of a linkage between the **I** and the $\mathbf{C_r}$ locus and take 30 per cent. as the preliminary recombination value.

The order of the three genes cannot be given with certainty, but most probably it is: **I**–(20 per cent.)–$\mathbf{B_2}$–(10 per cent.)–$\mathbf{C_r}$ and not $\mathbf{C_r}$–(30 per cent.)–**I**–(20 per cent.)–$\mathbf{B_2}$. There are indications of a linkage between $\mathbf{C_r}$ and $\mathbf{B_2}$ which are only to be expected on the first formula.

The factors for green flower colour and red flower pigment seem to be independent of each other and of all other factors.

[1] Prof. J. B. S. Haldane, using the method of maximum likelihood, found the same value as that estimated above.

One peculiar fact must, however, be mentioned in this connection. The four crassa plants which have appeared independently in different F_2 families were all characterised by the same flower coloration; they were all ivory (or possibly ivory on white) plus green, and had blue pollen. Their constitution must have been

$$\frac{(c)\ B_1\ i\ B_2\ G}{-\ -\ i\ -\ -},$$

which shows clearly that factors of three linkage groups were present. As yet I cannot explain this fact. That cytological aberrations may occur in these types, however, seems not impossible in view of the fact that different authors reported different chromosome numbers—Avery (1929), Ruttle (1927) 9, Kostoff (1929) 9 and Christoff (1928) 8. Experiments are in progress to test the various possible explanations.

From crosses, however, in which all four genes **c**, **i**, B_1 and B_2 were segregating, it may be concluded that between these four genes not more than the two linkages reported above can be detected.

Table VIII gives the results of selfing in the bud the triple heterozygotes $\frac{CB_1}{Cb_1}\frac{iB_2}{Ib_2}$. First considering the segregation of the genes in the second chromosome and assuming 20 per cent. of recombination, we should expect after selfing: 51 per cent. $I\text{–}B_2$,—24 per cent. $I\text{–}b_2b_2$, 24 per cent. iiB_2—and 1 per cent. iib_2b_2. Of the B_2 plants $\frac{3}{4}$ will also be B_1 and have blue pollen, while $\frac{1}{4}$ will [illegible] and be indistinguishable from b_2b_2 plants. Finally then we expect: 38·3 per cent. coloured blue, 36·7 per cent. coloured white, 18·0 per cent. ivory blue and 7 per cent. ivory white. The actual data are in very good agreement with this expectation, both in the individual families (cf. Table VIII) and in the total given below:

	Col. blue	Col. white	White-blue	White-white	Total
	%	%	%	%	
Found	43·6	36·3	13·4	6·7	
Expected	38·3	36·7	18·0	7·0	447
Standard error	2·3	2·3	1·8	1·2	
Deviation	+5·3	−0·4	−4·6	−0·3	

The results obtained after selfing plants heterozygous for five genes in the open flower gave the following results which are with one exception again in agreement with our linkage assumptions.

In the plants $\frac{S_f\ C\ B_1}{S_x\ c\ b_1}\frac{i\ B_2}{I\ b_2}$ we expect for the second chromosome genes the same segregation as given before. But here, on account of the

linkage between **S**, **C** and $\mathbf{B_1}$ and the eliminating effect of the $\mathbf{S_x}$ genes, 9/10 of the offspring receive the $\mathbf{C\,B_1}$ factors and only 1/10 the $\mathbf{b_1}$ allelomorphs. The expected figures are given below and compared with the figures found in the three larger families and in the total of all families (cf. also Table VII).

Number in family	Col. blue %	Col. white %	"White"-blue %	"White"-white %
152	46·0	27·0	9·9	17·1
133	47·4	24·8	9·0	18·8
63	58·7	20·6	19·1	1·6
Number in all families 396	50·3	24·2	10·9	14·6
Expected	45·9	21·6	21·6	10·9
Standard error	2·5	2·1	2·1	1·7
Deviation	+4·4	+2·6	−10·7	+3·7

In two of the families and in the totals there is a striking deficit in the class "white"-blue pollen, which I cannot yet explain. But otherwise the agreement with expectation is quite satisfactory considering the variation of the recombination values found in the individual cases and the possibly varying effect of the parasterility factors.

There are two more types of crosses segregating for all four or at least three loci in question. The crosses represent back-crosses for part of the factors and F_2 for others. The data given in Tables V and VI show a very close agreement with the expected values. All deviations are considerably smaller than even the simple standard error.

CONCLUSION.

The factorial analysis of the hybrids between *Nicotiana Langsdorffii* Weinm. and different types of the group *N. Sanderae* hort. gave results which are surprisingly simple, considering that these types differ so strikingly that they were originally placed in different sections of the genus.

The following genes have been identified:

(1) The white flower colour of some of the *Sanderae* lines is due to a recessive gene **c** which gives rise to absence of anthocyanin and related pigments in all organs such as stem, leaves, flowers, anthers and seeds. All other lines of *Sanderae* and *Langsdorffii* are **CC**.

(2) The ivory flower colour of the *alata* type within the *Sanderae* group, or of *Langsdorffii*, is due to another recessive factor **i**. All other types are **II**.

(3) White flower colour, **cc**, is epistatic over the other anthocyanin factors **R/r** and **I/i**.

(4) The red colour of the *Forgetiana* type within *Sanderae* is dominant

to magenta. In most cases this colour is due to one dominant gene **R**, while some families give more complicated results, indicating a bifactorial formula. The dominance of **R** is not always complete, and further the colour varies under the influence of external conditions. Most *Sanderae* types, including *alata*, and all *Langsdorffii* plants investigated carry the magenta gene **r**.

(5) The green flower colour of *Langsdorffii* is mainly due to one dominant gene **G**. All *Sanderae* types are **gg** and have very little or no chlorophyll. I have found one commercial type which diverged from typical *alata* in the deep green colour of the flowers.

(6) The blue pollen colour of *Langsdorffii* is due to two complementary genes $\mathbf{B_1}$ and $\mathbf{B_2}$. All *Sanderae* types have white pollen and are homozygous $\mathbf{b_1b_1b_2b_2}$.

(7) The character dark brown *versus* green anthers has not been fully analysed in the material reported on above. All **cc** plants had green anthers and apparently all **C** plants dark anthers. It is, however, possible that more factors are involved (cf. East, 1932).

(8) The **B** factors and the **c** gene do not interact so far as flower colour and pollen colour are concerned. The **B** factors have no effect on the first and the **cc** combination none on the second character. But the effect of **cc** on the seed coat colour and texture is hypostatic to that of the **B** factors. All blue pollen plants have brown and round seeds.

(9) A peculiar type of growth is described, characterised by very slow development, narrow strap-like leaves, and flowers though normal reduced in number. This crassa type appeared in several families of the *Langsdorffii-Sanderae* cross and is probably due to one recessive gene $\mathbf{c_r}$. All other lines seem normal $\mathbf{C_rC_r}$.

(10) The factors mentioned show the following linkage relations:

Chromosome I: **S**–(20 per cent.)–**C**–(0·5 per cent.)–$\mathbf{B_1}$.

Chromosome II: **I**–(20 per cent.)–$\mathbf{B_2}$–(10 per cent.)–$\mathbf{C_r}$.

Independent: **G** and **R**.

The position of the loci is probably the one given above, but the $\mathbf{B_1}$ and less probably the $\mathbf{B_2}$ genes may be on the left of the **C** and **I** loci.

(11) The segregation and expression of the factors studied shows no influence exerted by the cytoplasm. Only in the case of the green factor could this possibility not be entirely excluded, as all the segregating families had the cytoplasm of the non-green parent (*Sanderae*).

(12) The agreement between the figures found and the expectation is not always satisfactory, and shows definitely that factors modifying the ratios in various ways are present.

Note. While this paper has been in the course of publication, we have been able to investigate the cytology of some of the above-mentioned families. They show abnormalities which may explain the deviations mentioned in the text.

The pure *alata* line used as tester for the ivory gene fairly frequently shows not nine chromosome pairs, but eight pairs and two univalents, one of which very often lags and is not included in the daughter nuclei.

The F_1 between white *Sanderae* and *Forgetiana* giving an abnormal segregation for the *C* factor frequently has, instead of nine pairs, only five pairs and two groups of four chromosomes either in ring or chain association. The dissociation often seems to be non-disjunctional.

In both cases 97 per cent. of the pollen is good and only about 3 per cent. shrivelled.

Further and more detailed investigations are being carried out by Mr Sarup Singh and myself.

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EXPLANATION OF PLATE IV.

Fig. 1. Normal plant of *N. Sanderae* and crassa mutant.

Fig. 2. Offspring of crassa mutant after selfing.